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(54) **Lipase/acyltransferase from Candida parapsilosis**

(57) Polypeptides with lipase/acyltransferase activity are proposed with an amino acid sequence which is at least 49% identical to the sequence given in SEQ ID NO: 2, furthermore polypeptides which display this activity, furthermore nucleic acids (genes) which code for these polypeptides, vectors which contain nucleic acids which code for these polypeptides, transformed microorganisms which contain these nucleic acids, processes for the preparation of these polypeptides and also the use of the nucleic acids for the discovery of novel lipase/acyltransferases and the use of these lipase/acyltransferases as catalysts in chemical and biochemical processes.

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Description

Field of the invention

The present invention relates to polypeptides with lipase/acyltransferase activity, amino acid sequences of polypeptides which display this activity, nucleic acids (genes) which code for these polypeptides, vectors which contain nucleic acids which code for these polypeptides, transformed microorganisms which contain these nucleic acids, processes for the preparation of these polypeptides and also the use of nucleic acids to discover new lipase/acyltransferases and the use of these lipase/acyltransferases as catalysts in chemical and biochemical processes.

State of the art

During esterification according to the usual methods of chemical synthesis, on the one hand, due to the presence of several free hydroxyl groups in the alcoholic component or a partial ester thereof, mixtures of mono- and polysubstituted products can usually form with the result that the introduction and removal of protective groups is necessary if it is desired to synthesize a specific compound in a targeted manner.

Through the use of activated carboxylic acid derivatives, secondary products and often also undesired by-products form which make working-up more difficult, reduce the yields of desired product and harm the environment. These disadvantages can be avoided or at least reduced by carrying out the preparation enzymatically (e.g. according to the process described in the German application DE 197 53 789 B).

Enzymes are increasingly being used as catalysts in chemical and biochemical synthesis. Thus in many cases, due to the often milder reaction conditions,

hydrolases, in particular lipases (EC 3 1 1 3), are already being used for fat splitting in large-scale industrial processes.

Suitable enzymatic processes for esterification or transesterification are described for example in K Drauz and H Waldmann, Enzyme Catalysis in Organic Synthesis, VCH-Verlag, Weinheim 1975.

It is known that transesterification [can] be catalyzed by lipases in anhydrous media. If water is also present in the reaction system of esters, alcohols and lipases, a splitting of bonded fatty acids into free fatty acids normally takes place. As different lipases also catalyze the formation of esters from free fatty acids and alcohols, ultimately a transesterification reaction with an acid intermediate stage is carried out. For many industrial processes however it is a great disadvantage that free acids are formed in the system. In some cases, the water content prevents a technically and commercially acceptable conversion (development of a disadvantageous thermodynamic equilibrium). Costly technical systems (water removal via e.g. azeotropic distillation, membrane-separation processes, vacuum distillation) must be used to achieve satisfactory yields.

A thus far unique polypeptide which displays lipase/acyltransferase activity has already been described in the literature. This polypeptide was isolated from the microorganism *Candida parapsilosis* CBS 604. To date however, only the reactivity of this polypeptide has been assessed. With the polypeptide from the microorganism *Candida parapsilosis* which displays both lipase and acyltransferase properties, it is possible to keep the intermediate stage of a free (fatty) acid very low even in the presence of water (with an activity > 0.8). At the same time, the technical outlay can be kept within limits (cf. L. Vaysse, E. Dubreucq, J.-L. Pirat, P. Galzy; *J. Biotech* 53 (1997) 41-46).

The disadvantage with enzymatically catalyzed reactions often lies in the availability and stability of the polypeptides.

Description of the invention

The object of the present patent application is to characterize and make available polypeptides which make possible an acyl transfer in high yields even in an aqueous environment and thus overcome the customary disadvantages of a lipase-catalyzed esterification.

The object of the present invention is therefore to isolate these polypeptides, to decode the amino acid sequence and the nucleotide sequence which codes for this amino acid sequence, as these are essential both for the biotechnological preparation and for the further development of the polypeptide.

A further part-object is to make possible the biotechnological production of the found lipase/acyltransferase and thereby make them available in higher yields and then to have the possibility of discovering sequence homologies in alternative organisms via a screening. Included in this object is thus the provision of transformed host cells which are capable of producing this polypeptide.

By a polypeptide is meant in the context of the present application a polymer built up from natural amino acids, largely linear in structure and assuming a mostly three-dimensional structure to perform its function. In the present invention the 19 proteinogenous, naturally occurring L-amino acids are identified by the internationally customary 1- and 3-letter codes. Another name is also protein, wherein the number of monomer units in the polypeptide is to be at least 50.

Within the meaning of the invention, by "lipase/acyltransferase activity" is meant the activity of a polypeptide or enzyme which combines the properties of lipases with properties of acyltransferases. **Lipases** (EC 3 1 1 3) belong to the group of the hydrolases (specially the esterases) which specifically split fats (triglycerides) into glycerol and fatty acids; this process, called lipolysis, takes place at the

phase boundary between fat and water. An important property which leads to inclusion in the group of the hydrolases is the interfacial activity of lipases. Mechanistically a catalytic triad of serine, histidine and aspartic acid (or glutamic acid) plays a part in the catalysis. **Acyltransferases** (EC 2 3) are also called transacylases and belong to the group of the transferases. Very generally, they transfer acyl or in particular acetyl groups from a donor to an acceptor molecule and are therefore of particular importance in the build-up and breakdown of fats. Studies on the **lipase/acyltransferase** according to the invention have shown that this polypeptide is interfacially active and catalyzes reactions which are characteristic of lipases. It was also found that this polypeptide is capable of catalyzing transesterifications in the case of a water content in the reaction mixture which corresponds to a water activity of greater than 0.8. With this water content, a conventional lipase would catalyze only the hydrolysis of the esters. It is therefore a polypeptide which has features characteristic both of lipases and of acyltransferases. The naturally occurring polypeptide according to the invention is a lipase, due to sequence homologies to enzymes known to date such as for example lipase from *Candida albicans*, and an acyltransferase due to its enzymatic activity.

There can be used as preferred donors within the meaning of the invention for catalytic reactions with the lipase/acyltransferase according to the invention all possible esters, fats, triglycerides, 1,3-diglycerides, 1,2-diglycerides and 1-monoglycerides. There are used as preferred acceptors within the meaning of the invention for catalytic reactions with the lipase/acyltransferase according to the invention primary and secondary alcohols with one to five carbon atoms, in particular ethanol, propanol, butanol, 1,2-propanediol, 1,3-propanediol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and hydroxyl amines.

The expression "identity" used here in relation to the amino acid sequence denotes a homology to the given amino acid sequence which has the effect that the polypeptide with a given identity has the same biological activity as the first

polypeptide. The identity of the nucleotide sequence relates to a gene homologous to a first nucleotide sequence. Homologous means in relation to the nucleotide sequence that the gene can be allelic. Homologous also means that the gene can come from a different species, but the polypeptide coded for by this gene has the same biological activity as the polypeptide coded for by the first nucleotide sequence.

The subject of the invention comprises polypeptides with lipase/acyltransferase activity with an amino acid sequence which have at least 49%, preferably 80%, preferably at least 98%, particularly preferably 99.8% and in particular 100% identity with the amino acid sequence given in SEQ ID NO: 2. Identity of at least 96% to the amino acid sequence given in SEQ ID NO: 2 applies in particular to the region which corresponds to the amino acids in positions 190 to 390. A polypeptide which has 100% identity with the amino acid sequence given in SEQ ID NO: 2 contains 465 amino acids. Particularly preferred is a 96% identity for the regions in positions 190-200, 220 to 290 and 330 to 385, in particular for positions 196, 240 and 361.

The amino acid sequence of the enzyme according to the invention is identified in the sequence listing as SEQ ID NO: 2. The nucleotide sequence of this enzyme is given in the sequence listing under the name SEQ ID NO: 1. It is thus available for further developments via molecular biological methods known per se.

Comparable polypeptides with lipase/acyltransferase activity are represented by likewise preferred versions of the present invention and are claimed inasmuch as they have amino acid and/or nucleic acid sequences which lie within the ranges of similarity to the sequences given in SEQ ID NO: 1 and/or SEQ ID NO: 2. This range of similarity includes all polypeptides the amino acid sequence of which has at least 49%, 80%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, 99.8% or 100% identity with the amino acid sequence given in SEQ ID NO: 2.

This applies in particular to those regions of the protein which pertain to the amino acids 190 to 390.

A polypeptide which has at least 80% identity with the amino acid sequence given in SEQ ID NO: 2 has a molecular mass between 49 and 55 kD after deglycosylation, in particular 54 kD. The pH optimum for the catalytic reaction of transesterification, hydrolysis or esterification which was determined at 28°C is between 3 and 8.5, preferably between 4 and 8, in particular between 6 and 7.5.

An optimum temperature range during the catalysis of the hydrolysis, determined at the pH optimum, lies between 30 and 50°C, preferably between 35 and 40°C. An optimum temperature range for the catalysis of the transesterification and the esterification, determined at the pH optimum, lies between 20 and 50°C, preferably between 20 and 30°C.

The polypeptides according to the invention also include enzymes which are sufficiently similar to these or can be derived with methods known per se.

In a particular version of the invention, the polypeptides with lipase/acyltransferase activity are present glycosylated with an amino acid sequence which has at least 49%, preferably 80%, preferably at least 98%, particularly preferably 99.8% and in particular 100% identity with the amino acid sequence given in SEQ ID NO: 2. The positions at which the polypeptide is present glycosylated and the degree of glycosylation depend on the organism which produces this polypeptide. A degree of glycosylation of 1 to 2 sugar radicals per molecule of polypeptide is preferred.

In a further version of the invention, the polypeptides are linked to a further peptide. Such a peptide can be a marker which can for example result in being able to more effectively purify the desired polypeptide in chromatography, in particular affinity chromatography. According to the invention, the further

polypeptide is the his-tag marker. His-tag is a peptide which is built up from six monomer histidine units.

A particular version of the invention comprises polypeptides with lipase/acyltransferase activity with an amino acid sequence which have at least 49%, preferably 80%, preferably at least 98%, particularly preferably 99.8% and in particular 100% identity with the amino acid sequence given in SEQ ID NO: 4.

A further preferred version of the invention comprises polypeptide fragments or polypeptides obtainable by deletion mutation with a lipase/acyltransferase activity according to the polypeptides described above.

By **fragments** are meant all proteins or peptides which are smaller than natural proteins which are smaller than those proteins which correspond to those of SEQ ID NO: 1 or SEQ ID NO: 2 or SEQ ID NO: 3 or SEQ ID NO: 4, but are sufficiently homologous to them in the corresponding part-sequences, or those which correspond to fully translated genes and for example can also be obtained synthetically. Due to their amino acid sequences they can be allocated to the complete proteins concerned. For example, they can assume identical structures or perform proteolytic activities or partial activities such as for example the complexing of a substrate. Fragments and deletion variants of starting proteins are in principle similar; while fragments are more likely to represent smaller pieces, the deletion mutants are more likely to lack only short regions, and thus only individual part-functions.

The fragments can for example be individual domains or pieces which do not match the domains. Such fragments can be more cheaply produced, no longer have certain possibly disadvantageous characteristics of the starting molecule, such as possibly an activity-reducing regulation mechanism, or display a more favourable activity profile. Such protein fragments can also be prepared non-

biosynthetically, but for example chemically. Chemical synthesis can for example be advantageous if chemical modifications are to be made after the synthesis.

Because of their basic similarity, polypeptides obtainable by deletion mutation can also be included among the fragments. These can be largely the same as the starting molecules biochemically or actually no longer have individual functions. This seems particularly useful for example in the deletion of inhibiting regions. As a result, the deletions can be accompanied by both a specialization and a broadening of the application field of the protein. Inasmuch as, in the widest sense, lipase/acyltransferase activity is thereby maintained, modified, made more specific or even just achieved, the deletion variants, like the fragments, are proteins according to the invention; the only additional precondition is that, in addition to the still present homologous part-sequence, they lie within the given range of similarity to the sequences SEQ ID NO: 1 and SEQ ID NO: 2 and SEQ ID NO: 3 and SEQ ID NO: 4.

A further version of the invention comprises polypeptides obtainable by insertion mutation or chimeric polypeptides with lipase/acyltransferase activity which are at least partly composed of a polypeptide which is identical to a polypeptide or fragment described above.

By **chimeric** or hybrid polypeptides are meant within the meaning of the present application proteins which are composed of elements which come naturally from different polypeptide chains from the same organism or from different organisms. This procedure is also called shuffling or fusion mutagenesis. The purpose of such a fusion can be for example to effect or modify a specific enzymatic function with the help of the fused protein section. It is therefore immaterial within the meaning of the present invention whether such a chimeric protein consists of a single polypeptide chain or several sub-units over which various functions can be distributed. To realize the last-mentioned alternative it is for example possible to separate a single chimeric polypeptide chain into several, post-translationally or

only after a purification step, by a targeted proteolytic splitting. The subject of the invention also includes chimeric proteins which, due to their structure, have beyond their whole amino acid and/or nucleotide sequence a possibly lower identity than defined for the similarity range according to the invention, which can however be attributed to it in at least one of the regions introduced by fusion, and in this region perform the same functions as in a lipase/acyltransferase which comes under the named homology range over its whole length.

By polypeptides obtained by **insertion mutation** are meant those which have been obtained using methods known per se by insertion of a nucleic acid fragment or protein fragment into the starting sequences. They are to be included among the chimeric proteins on account of their basic similarity. They differ from them merely in the size ratio of the unmodified protein section to the size of the whole protein. In such insertion-mutated proteins, the foreign protein content is smaller than in chimeric proteins.

Inversion mutagenesis, i.e. a partial sequence reversal, can be regarded as a special form both of deletion and insertion. The same applies to a regrouping of different molecule parts which deviates from the original amino acid sequence. They can be regarded as a deletion variant, as an insertion variant, and also as a shuffling variant of the original protein.

A further version of the invention comprises derivatives of a polypeptide with lipase/acyltransferase activity according to one of the polypeptides described above.

By **derivatives** are meant within the meaning of the present application polypeptides, the pure amino acid chain of which has been chemically modified. Such derivatizations can take place for example biologically in conjunction with the protein biosynthesis by the host organism. For this, molecular biology methods can be used. But they can also be carried out chemically, say by the

chemical conversion of a side chain of an amino acid or by covalent bonding of a different compound to the protein. Such a compound can for example be other proteins which are for example bonded to polypeptides according to the invention via bifunctional chemical compounds. By derivatization is likewise meant the covalent bonding to a macromolecular carrier. Such modifications can for example influence the substrate specificity or the bonding strength to the substrate or bring about a temporary blocking of the enzymatic activity if the coupled substance is an inhibitor. This can be useful for example for the storage period. A further version therefore comprises derivatives which have been obtained by covalent bonding to a macromolecular carrier, such as for example polyethylene glycol or a polysaccharide.

Within the meaning of the present invention all polypeptides, enzymes, proteins, fragments and derivatives, provided they do not need to be explicitly referred to as such, are included in the generic term polypeptides.

The enzymatic activity can be qualitatively or quantitatively modified by other regions of the polypeptide which are not involved in the actual reaction. This applies for example to the enzyme stability, the activity, the reaction conditions or the substrate specificity. On the one hand it is not precisely known which amino acid radicals of the polypeptide according to the invention actually catalyze the hydrolysis, transesterification and esterification, and on the other hand individual functions not determined in advance cannot be definitively excluded from participation in the catalysis. The auxiliary functions or partial activities include for example the bonding of a substrate, intermediate or end-product, the activation or inhibition or engineering of a regulating influence on the hydrolytic activity. For example, this can also be the development of a structural element which lies far from the active centre, or a signal peptide the function of which relates to the transfer of the formed protein from the cell and/or its correct folding and without as a rule a functional enzyme being formed *in vivo*. However, overall a hydrolysis, transesterification and esterification must be catalyzed.

A further achievement of the object according to the invention comprises polypeptides or derivatives which have at least one antigenic determinant in common with one of the abovenamed polypeptides or derivatives.

Decisive for the performance of enzymatic activities is not alone the pure amino acid sequence of a protein, but also its secondary structural elements and its three-dimensional folding. Thus domains which deviate clearly from one another in their primary structure can develop spatially largely identical structures and thus make identical enzymatic behaviour possible. Such common features in the secondary structure are usually recognized as corresponding antigenic determinants of antisera or pure or monoclonal antibodies. Structurally similar proteins or derivatives can thus be detected and allocated via immunochemical cross-reactions.

Therefore, precisely those polypeptides or derivatives which display lipase/acyltransferase activity and can be included in the above-defined proteins or derivatives according to the invention, possibly not via their homology values in the primary structure, but surely via their immunochemical affinity, are also included within the scope of protection of the present invention.

Polypeptides according to the invention which come from natural sources are preferred versions of the present invention, in particular if they come from microorganisms such as monocellular fungi or bacteria. These are usually easier to handle than multicellular organisms or the cell cultures derived from multicellular organisms. These represent useful options for special versions.

Polypeptides or derivatives according to the invention from eukaryotic fungi, in particular those which can release secreted proteins directly into the surrounding medium, are particularly preferred.

Quite particularly preferred are polypeptides or derivatives according to the invention which can be obtained from microorganisms which are selected from the group which is formed by *Candida parapsilosis* and preferably *Candida parapsilosis CB 604, Candida antarctica (Trychosporon oryzae, Pseudozyma antarctica), Candida glabrata, Candida albicans, Candida maltosa, Candida tropicalis, Candida viswanathil, Issatchenkia orientalis (Candida krusei), Kluyveromyces marxianus (C. kefyr, C. pseudotropicalis), Pichia guilliermondii (Candida guilliermondii), Geotrichum candidum, Fusarium solani* and *Aeromonas aerophila.*

Among the polypeptides according to the invention or derivatives from Candida species, again those from *Candida parapsilosis* are preferred, among them in particular from *Candida parapsilosis CBS 604*, because the version of the enzyme according to the invention, the sequences belonging to which are given in the sequence listing, was originally obtained from this.

For production reasons, strains which release the formed polypeptide into the medium surrounding them are preferred in each case.

A further subject of the invention comprises nucleic acids which code for a polypeptide with lipase/acyltransferase activity, the nucleotide sequence of which is 100% identical to the nucleotide sequence given in SEQ ID NO: 1, in particular over the region which corresponds to the amino acids 190 to 390 according to SEQ ID NO: 2. Furthermore nucleic acids are claimed which code for an amino acid sequence which has at least 49%, preferably 80%, particularly 99.8% and in particular 100% identity with the amino acid sequence given in SEQ ID NO: 2. Nucleic acids are preferred which code for amino acid sequences which are at least 96% identical to the amino acid sequence given in SEQ ID NO: 2 in positions 190 to 390. Nucleic acids are particularly preferred which code for one of the polypeptides or derivatives described above. The similarity range also includes all polypeptides the nucleotide sequence of which is at least 85%,

87.5%, 90%, 92.5%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence given in SEQ ID NO: 1.

A further subject of the invention comprises nucleic acids which code for a polypeptide with lipase/acyltransferase activity, the nucleotide sequence of which is identical to the nucleotide sequence given in SEQ ID NO: 3.

Also included in the scope of protection are nucleic acids coding for an amino acid sequence which has at least 49%, preferably 80% identity with the amino acid sequence given in SEQ ID NO: 4. The similarity range also includes all polypeptides the nucleotide sequence of which is at least 85%, 87.5%, 90%, 92.5%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence given in SEQ ID NO: 3.

By nucleic acids are meant for the purpose of the present application the molecules, built up naturally from nucleotides serving as information carriers, which code for the linear amino acid sequence in proteins or enzymes. They can be present as a single strand, as a single strand complementary to this single strand or as a double strand. As the naturally longer-lasting information carrier, the nucleic acid DNA is preferred for molecular biology work. On the other hand, to realize the invention in a natural environment, such as for example in an expressing cell, an RNA is formed, which is why RNA molecules essential to the invention also represent versions of the present invention.

With DNA, the sequences of both complementary strands are to be considered in each of all three possible reading frames. It is also to be considered that different codon triplets can code for the same amino acids with the result that a specific amino acid sequence can be derived from several different nucleotide sequences possibly having only low identity (degeneracy of the genetic code). In addition, different organisms display differences in the use of this codon. For these reasons, both amino acid sequences and nucleotide sequences must be included

when considering the scope of protection, and given nucleotide sequences are in each case to be regarded only as an example of coding for a specific amino acid sequence.

The information unit corresponding to a protein is also called a gene in the context of the present invention.

It is possible for a person skilled in the art, using methods generally known today, such as for example chemical synthesis or polymerase chain reaction (PCR) in conjunction with standard methods in molecular biology and/or protein chemistry, to prepare the corresponding nucleic acids up to complete genes using known DNA and/or amino acid sequences. Such methods are known for example from the "Lexikon der Biochemie", Spektrum Akademischer Verlag, Berlin, 1999 Volume 1, pp. 267-271 and Volume 2, pp. 227-229.

Changes in the nucleotide sequence, such as can for example be effected by molecular biology methods known per se, are called mutations. Depending on the nature of the change, deletion, insertion or substitution mutations are known for example, or those in which different genes or parts of genes are fused (shuffling); these are gene mutations. The associated organisms are called mutants. The proteins derived from mutated nucleic acids are called variants. Thus for example deletion, insertion, substitution mutations or fusions lead to deletion-, insertion-substituted-mutated or fusion genes and at protein level to corresponding deletion, insertion or substitution-variants, or fusion proteins.

A further achievement of the object according to the invention and thus a particular subject of the invention are the organisms which naturally form a protein or derivative according to the invention or contain nucleic acids which code for a polypeptide or derivative according to the invention. Discovering them makes it possible to implement the idea of the invention. Such organisms can be obtained using generally known techniques, for example by isolating strains from

a natural habitat or by screening gene banks. The nucleotide sequence given in SEQ ID NO: 1 can be used for example as a probe for screening or serve as a template for the design of corresponding PCR primers. Analogously thereto, short-chained or complete peptides with amino acid sequences according to SEQ ID NO: 2 can be used to form corresponding antisera with the help of which corresponding organisms, or the proteins released by them, can be identified.

Corresponding to that stated above, microorganisms are preferred because they are established in industrial processes primarily on the grounds of cultivatability and as production organisms with a particularly high production capacity, preferably yeast fungi, including those of the Candida genus, in particular *Candida parapsilosis* and quite particularly *Candida parapsilosis CBS 604*.

For production reasons, strains which release the formed polypeptide into the medium surrounding them are preferred in each case.

It is possible that, although naturally occurring producers can prepare an enzyme according to the invention, they can express it and/or release it into the surrounding medium to only a small extent under the conditions initially ascertained. Nevertheless they come within the scope of protection of the present invention as long as it is possible to experimentally ascertain suitable environmental conditions or lower-molecular or other factors under the action of which they can be incited to produce the protein according to the invention such that its economic use seems sensible. Such a regulation mechanism can be used in a targeted manner for biotechnological production, for example for regulating the promoters responsible.

Depending on whether a protein has been isolated, worked up or prepared, it can be associated with various other substances, in particular if it has been isolated from natural producers of this protein. It can then, but also independently thereof, be reacted in targeted manner with certain other substances, for example to

increase its storage stability. By protein according to the invention is therefore also meant in addition all preparations of the actual protein essential to the invention. This is also irrespective of whether it actually does or does not display this enzymatic activity in a specific preparation. It may be desirable that it has no, or only little, activity during storage and performs its function only at the time of use. This can for example depend on the folding state of the protein or result from the reversible bonding of one or more companion substances from the preparation or from another control mechanism.

Nucleic acids form the starting point for molecular biology studies and further developments. Such methods are described for example in the manual by Pritsch, Sambrook and Maniatis "Molecular cloning: a laboratory manual", *Cold Spring Harbour Laboratory Press*, New York, 1989. All the genetic engineering and protein-biochemical methods included under the concept of protein engineering in the state of the art are also gene-based, in particular on the cloned gene. With these, polypeptides according to the invention can be further optimized with a view to different uses, for example by point mutagenesis or by fusion with sequences from other genes.

Vectors which contain one of the described nucleic acid regions which code for a polypeptide with lipase/acyltransferase activity according to the invention are themselves a subject of the invention.

To handle nucleic acids, the DNA is cloned in a suitable way into a vector. Vectors are DNA molecules which, as transport molecules (vehicles), are suitable for introducing (transforming) foreign DNA into host cells and if necessary are autonomously replicable there. Frequently used vectors are plasmids, i.e. extrachromosomal, annular, double-stranded bacterial DNA which can be introduced by suitable methods into other microorganisms and multiplied there.

Vectors include for example those which derive from bacterial plasmids, from viruses or from bacteriophages, or predominantly synthetic vectors or plasmids with elements from the most varied sources. With the other genetic elements available in each case, vectors can establish themselves as stable units in the host cells concerned over several generations. It is therefore irrelevant for the purpose of the invention whether they establish themselves extrachromosomally as separate units or integrate to form a chromosome. Which of the numerous systems known from the state of the art is chosen depends on the individual case. Decisive can be for example the achievable number of copies, the selection systems available, including above all resistances to antibiotics, or the cultivatability of the host cells capable of receiving the vectors.

Vectors form suitable starting points for molecular biology and biochemical studies of the gene concerned or associated protein and for further developments according to the invention and finally for the amplification and production of proteins according to the invention. They thus represent versions of the present invention inasmuch as the sequences of the contained nucleic acid regions according to the invention lie within the homology region described in more detail above.

Preferred versions of the present invention are cloning vectors. These are suitable, in addition to storage, for the biological amplification or selection of the gene of interest for the characterization of the gene concerned, say by creating a restriction map or sequencing. Cloning vectors are also preferred versions of the present invention because they represent a transportable and storable form of the claimed DNA. They are also preferred starting points for molecular biology techniques which are not bound to cells, such as for example the polymerase chain reaction.

Expression vectors have part-sequences which enable them to replicate in host organisms optimized for the production of proteins and to express the gene

contained there. Preferred versions are expression vectors which themselves carry genetic elements necessary for expression. The expression is influenced for example by promoters which regulate the transcription of the gene. Thus the expression can take place through the natural promoter originally located by this gene, but also after genetic fusion both by a promoter, prepared on the expression vector, of the host cell and by a modified or completely different promoter of a different organism.

Preferred versions are expression vectors which can be regulated via changes in the culture conditions or by adding specific compounds, such as for example the cell density or special factors. Expression vectors make it possible for the associated protein to be produced heterologously, i.e. in an organism other than that from which it can be naturally obtained. A homologous protein isolation from a host organism naturally expressing the gene via a suitable vector lies within the scope of protection of the present invention. This can have the advantage that natural modification reactions related to the translation can be carried out on the resultant protein exactly as they would occur naturally.

To isolate the polypeptides according to the invention, microorganisms are cultivated which have been transformed by an expression vector which contains the structural gene coding for the corresponding enzyme. The expression vectors have been obtained in this case by processes described further below. The particularly preferred microorganisms which are transformed by the expression vector are *Saccharomyces cerevisiae* and *Pichi pastoris*. Preferred vectors are plasmids the restriction maps of which are shown in **Figures 1 to 3**.

The vectors used within the framework of the invention include those which are formed by cutting with suitable restriction endonucleases, preferably *BamHI* or *SnaBI* and subsequent recombination with the corresponding N- or C-terminal halves of the enzyme structural gene. Restriction endonucleases are enzymes which break substrate-specific double-stranded DNA down into fragments by

splitting phosphate diester bonds between individual nucleotide components of DNA. All restriction endonucleases can recognize specific base sequences of DNA which mark specific action sites (cutting sites) for the activity of the restriction endonucleases concerned. When cutting (restricting) double-stranded DNA, specific so-called "sticky ends" form with some restriction endonucleases, which under specific renaturation conditions can be joined (ligated) together again or to corresponding (complementary) sticky ends of DNA fragments obtained in some other way (recombination).

Versions of the present invention can also be cell-free expression systems in which the protein biosynthesis is completed *in vitro*. Such expression systems are likewise established in the state of the art.

A further version of this subject of the invention is represented by cells which contain one of the vectors defined above, in particular a cloning or an expression vector. In particular in the course of molecular biology work, such as is necessary for example for mutagenesis, sequencing or storage of the vectors, their transformation into corresponding cells takes place. For example gram-positive, but in particular also gram-negative bacteria are suitable for this, depending on the method.

A further version comprises host cells which express or can be incited to express polypeptide or derivative of the first subject of the invention, preferably using one of the expression vectors defined above.

The preferred *in-vivo* synthesis of a polypeptide according to the invention requires the transfer of the associated gene into a host cell. All organisms, i.e. prokaryotes, eukaryotes or cyanophyta, are suitable in principle as host cells. Host cells are preferred which can be handled well genetically, which concerns for example transformation with the expression vector and its stable establishment, for example monocellular fungi or bacteria. Moreover, preferred

host cells are characterized by a good microbiological and biotechnological manageability. This relates for example to easy cultivatability, high growth rates, low fermentation media requirements and good production and secretion rates for foreign proteins. The optimum expression systems for the individual case must often be established experimentally from the wealth of different systems available according to the state of the art. Each protein according to the invention can be isolated in this way from a large number of host organisms.

Preferred versions are represented by host cells which, due to genetic regulation elements which are for example made available on the expression vector, but can also be present in these cells from the outset, can be regulated in their activity. For example, these can be incited to expression by controlled addition of chemical compounds, such as for example methanol, which serve as activators, by changing the cultivation conditions or upon reaching a specific cell density. This makes possible a very economic production of the proteins of interest.

A variant of this test principle is represented by expression systems in which additional genes, for example those which are made available on other vectors, influence the production of proteins according to the invention. These can be modifying gene products or those which are to be purified together with the protein according to the invention, say in order to influence its function. These can be for example other proteins or enzymes, inhibitors or elements which influence the interaction with various substrates.

Preferred host cells are prokaryotic or bacterial cells. Bacteria differ from eukaryotes as a rule by virtue of shorter generation times and lower demands as regards the cultivation conditions. Cost-favourable processes for isolating proteins according to the invention can thereby be established.

Host cells, in particular bacteria, are particularly preferred which secrete the formed protein or derivative into the surrounding medium, with the result that the expressed proteins according to the invention can be purified directly.

Heterologous expression is preferred. Gram-positive bacteria such as for example actinomyctenes or bacilli have no outer membrane with the result that they release secreted proteins directly into medium surrounding them. Bacteria preferred for heterologous expression thus include those of the genus bacillus, in particular those of the species which are listed below.

Gram-negative bacteria can also be used for heterologous expression. With these, a large number of proteins are secreted into the periplasmic space, i.e. into the compartment between the two membranes enclosing the cells. This can be advantageous for special applications. These include for example those of the genera Klebsiella or Escherichia, preferably the species which are also listed below.

Eukaryotic cells can also be suitable for the production of polypeptides according to the invention. Examples of this are yeasts such as *Saccharomyces* or *Kluyveromyces*. This can be particularly advantageous for example if the proteins are to experience specific modifications in connection with their synthesis which make such systems possible. These include for example the bonding of low-molecular compounds such as membrane anchors or oligosaccharides.

Particularly preferred for the production of polypeptides according to the invention from transformed host cells are microorganisms which are selected from the group which is formed by *Candida parapsilosis, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris, Pichia boldini, Pichia stipitis, Hansenula polymorpha, Kluyveromyces lactis, Schwanniomyces castellii, Yarrowia lipolytica, Escherishia coli, Bacillus sublilis, Bacillus amylolichefaciens, Bacillus stearothermophilus, Bacillus licheniformis, Lactococcus lactis,*

Streptocuccus lactis, Lactobacillus bulgaricus, Aspergillus orizae, Aspergillus niger, Trichoderma reesei, Mucor sp and *Rhizopus sp.*

The transformed host cells, also called transformants, are then cultivated in a manner known per se, preferably as described in the examples, and the formed polypeptides according to the invention isolated.

All elements already listed above can be combined to form processes for the preparation of polypeptides according to the invention. These processes therefore represent a further subject of the invention. A large number of possible combinations of process steps are conceivable for any protein according to the invention. They all put into practice the idea underlying the present invention, namely the quantitative preparation with the help of the associated genetic information of representatives of a protein type defined via the lipase/acyltransferase activity and simultaneously the high homology to the sequences given in the sequence listings. The optimum process must be ascertained experimentally for each specific individual case.

In principle, the procedure is as follows: nucleic acids according to the invention, i.e. those which lie within the range of similarity, defined above, to the sequence of SEQ ID NO: 1 or SEQ ID NO: 3, are ligated in suitable manner in the form of DNA into a suitable expression vector. This is transformed into the host cell, for example into cells of an easily cultivated bacterial strain which transfers the proteins, the genes of which are under the control of corresponding genetic elements, into the surrounding culture medium; regulating elements for this can for example be made available by the expression vector. The protein according to the invention can be purified from the surrounding medium over several purification steps, such as for example precipitations or chromatographies. A person skilled in the art is able to transfer a system which has been experimentally optimized on a laboratory scale to an industrial production scale.

A further subject of the invention is the use of natural and/or recombinant microorganisms as described above, containing a nucleic acid for the preparation of a polypeptide, described above, according to the invention.

Further use according to the invention of the nucleic acids described above and/or amino acid sequence which have at least 49%, preferably 80%, preferably at least 98%, particularly preferably 99.8% and in particular 100% identity with the amino acid sequence given in SEQ ID NO: 2 and/or in SEQ ID NO: 4 [is] for the discovery of new acyltransferases.

This discovery of new enzymes is also called screening. In particular gene banks of specific organisms are screened using general methods such as are given for example in Fritsch, Sambrook and Maniatis "Molecular cloning: a laboratory manual", *Cold Spring Harbour Laboratory Press*, New York, 1989.

Through comparison with known enzymes which are stored for example in generally accessible databases, characteristic molecule parts such as for example structural elements or the enzymatic activity of an observed enzyme can be deduced from the amino acid or nucleotide sequence. Such a comparison is carried out by assigning similar sequences in the nucleotide or amino acid sequences of the observed proteins to one another. This is called homologization. A tabular assignment of the positions concerned is called alignment. In the analysis of nucleotide sequences, both complementary strands of each of all three possible reading frames are again to be taken into account; likewise the degeneracy of the genetic code and the organism-specific codon usage. Alignments are now produced using computer programs such as for example by the FASTA or BLAST algorithms; this procedure is described for example by D. J. Lipman and W. R. Pearson (1985) in *Science*, Volume 227, p. 1435-1441. A combination of all the matching positions in the compared sequences is called a consensus sequence.

Such a comparison also allows a statement to be made about the similarity or homology of the compared sequences to one another. This is expressed in percentage identity, i.e. the proportion of identical nucleotides or amino acid radicals at the same positions. A broader concept of homology also includes the conserved amino acid exchanges in this value. The term used then is percentage similarity. Such statements can be made about complete proteins or genes or about individual regions only.

Homologous regions of different proteins are mostly those with identical structural elements and/or functions which can be recognized by matches in the primary amino acid sequence. It goes up to full identities in the smallest regions, so-called boxes, which comprise only a few amino acids and mostly perform functions which are essential for the overall activity. By functions of the homologous regions are meant very small part-functions of the function performed by the whole protein, such as for example the development of individual hydrogen bridge bonds for complexing a substrate or transition complex.

On the basis of alignments, largely the same secondary and tertiary structures can be assumed for polypeptides according to the invention as for the proteins used for homologization. Their structural elements can be retrieved in generally accessible databases such for example at the EMBL European Bioinformatics Institute (EBI) in Cambridge, United Kingdom (http://www.ebi.ac.uk), Swiss-Prot or GenBank (National Center for Biotechnology Information NCBI, National Institutes of Health, Bethesda, MD, USA). Should structures differing from this result, or should it transpire that different folding variants with varying properties exist, which concerns for example the optimum reaction conditions or the substrate specificity, these are all included in the scope of protection of the present invention. Firstly, the folding can depend on the preparation conditions, for example the presence of absence of the leader peptide. Secondly, these

variants can prove to be particularly suitable for different possible uses in each case.

A further subject of the invention is the use of described polypeptides as catalysts in acyl transfer reactions, in particular in reactions which are selected from the group which is formed by alcoholysis of esters, in particular of glycerols or sterols, alcoholysis of thioesters, thiolysis of esters, aminolysis of an ester with hydroxylamines or hydrazines; reaction of an ester with hydrogen peroxides and enantioselective synthesis of esters, thioesters or lactones by alcoholysis. Special reactions which are catalyzed by the polypeptides according to the invention are described for example in: a) Fournand et al J Mol Catalysis B, 1998, 5. 207-211; b) Briand et al Eur J Biochem 1995, 228, 169-175.

Examples

Example 1: Cultivation of strain and isolation of polypeptide

The *Candida parapsilosis* strain (Ashford) Langeron and Talice, CBS 604, was deposited with the Centraalbureau voor Schimmelcultures, Yeast Division, Delft, Netherlands.

Culture was carried out as described by Briand et al in *Eur J Biochem*, 1995, 228, 169-175. The main culture was adjusted to a pH of 6.5 with 100 mM of phosphate buffer and supplemented with 5 g/l of glucose as the C source.

At the end of the exponential growth phase, the culture medium was centrifuged (7000 g for 15 min) and the lipase/acyltransferase was obtained from the liquid supernatant. The polypeptide was purified using the method described by Riaublanc A et al in *J Am Oil Chem Soc* 1993, 70, 497-500.

Example 2: Molecular biological operational steps

All molecular biological operational steps follow standard methods as described, for example, in handbooks such as that by Fritsch, Sambrook and Maniatis, "Molecular Cloning: A Laboratory Manual", *Cold Spring Laboratory Press*, New York, 1989.

The lipase/acyltransferase content was measured in units (U), measured as the amount of oleic acid obtained per minute when trioleylglycerol is hydrolyzed under the conditions described by Briand et al in *Eur J Biochem* 1995; 228; 169-175. The protein concentration was determined using the method described by Bradford (1976; *Anal Biochem* 72; 248-254).

Example 3:Expression of a gene containing the nucleic acid of SEQ ID NO: 1 in *Saccharomyces cerevisiae*

In order to express the desired nucleic acid sequence, DNA was initially partially hydrolyzed with the restriction endonuclease *Bam*HI. Primers were constructed using degenerate PCR, which primers contained the nucleic acid of SEQ ID NO: 1. The following primer pairs were used (the start and stop codons are underlined; the BamHI-restriction site is shown in bold):

forward 5'-CTC**GGATCC**ATGCGTTACTTTGCTATTGC

reverse 5'-CAC**GGATCC**TTAAAAAGCAAAACGTTCCAACTTGAGCAATCC

The following time/temperature program was followed for PCR amplification: denature 5 min at 95°C; subsequently 30 cycles of 1 min at 95°C, 1 min at 50°C, 1 min at 72°C and as the final step, 10 min at 72°C.

The fragments from PCR were digested with the restriction endonuclease *Bam*HI and then ligated into the vector pVT100-U cleaved with the restriction endonuclease *Bam*HI to produce a plasmid. The vector pVT100CpLIP2 of **Figure 1** (replicative plasmid) was obtained. The absence of mutations was checked by sequencing the insert. Transformation of the newly combined DNA in the *Saccharomyces cerevisiae* W303-1a strain was carried out using the electroporation method described by Becker et al in *Methods in Enzymology*, 1991, 194, 182-187. The transformants were selected on YNB medium lacking uracil (6.7 g/l Yeast Nitrogen Base lacking amino acid from [Difco], 20 g/l of glucose, 150 mg/l leucine, 100 mg/l adenine, 100 mg/l histidine, 100 mg/l tryptophan) at a frequency of 1-2 x 10^5 transformants per µg of DNA. The transformants were selected in a lipase activity plate assay using Kouker's method as described in: Kouker G et al, *Applied Environ Microbiol* 1987, 59, 211-213.

The selected transformant was cultivated in a shaker flask at 28°C in YPD medium (YPD = 10 g/l yeast extract [Difco], 20 g/l Bacto-peptone [Difco], 60 g/l glucose, 150 mg/l leucine, 100 mg/l adenine, 100 mg/l histidine and 100 mg/l tryptophan). The culture medium was harvested after 36 hours fermentation and the supernatant of the culture solution was separated from the residue by centrifuging. The supernatant contained 2500 U of the recombinant lipase/acyltransferase per litre and had a specific activity of 0.7 U/mg. After concentrating by ultrafiltration and hydrophobic chromatography on phenylsepharose 6 Fast-Flow Gel, 10% of the activity was recovered with a specific activity of 80 U/mg.

Example 4: Expression of a gene containing SEQ ID NO: 1 in *Pichia pastoris*

The lipase/acyltransferase was expressed as a fusion to an N-terminal peptide which encodes the secretion signal for the α-factor from *Saccharomyces cerevisiae*. Initially, the gene corresponding to SEQ ID NO: 1 was amplified by PCR to thereby produce a cleaved gene of the mature gene. The following primers were used: (the stop codon is underlined; the first phenylalanine codon of the mature gene is shown in bold)

forward 5'-**TTT**GTCTTGGCTCCCAAAAAGCCA

reverse 5'-TTAAAAAGCAAAACGTTCCAACTTGAGCAATCC

The following time/temperature program was followed for the PCR amplification: denature 2 min at 94°C; then 15 cycles of 15 sec at 94°C, 30 sec at 50°C, 90 sec at 72°C, plus 5 sec per cycle for the extension period from cycle 11; with a final step of 7 min at 72°C.

After amplification, the fragment obtained was phosphorylated with T4-polynucleotide-lipase and blunted with T4-DNA-polymerase. The fragment was

then ligated with a pPIC9K plasmid digested with *Sna*BI to obtain the vector pPIC9KCpLIP2 (replicative plasmid) (**Figure 2**). The absence of mutations was checked by sequencing the insert.

The yeast spheroplasts were transformed using the *Pichia* Expression Kit from Invitrogen (Groningen, Netherlands). The transformation frequency was 10^3 transformations per µg of DNA.

A selected transformant was supplemented with a synthetic medium described by Boze et al in: Boze H et al; *Process Biochem* 2001, 36, 907-913) to which 40 g/l glycerol was added, cultivated in a fermenter. After the growth phase (following 2500 min fermentation) in the batch method, using the fed-batch method, pure methanol (5 g/l) was added to induce expression of the gene. After cultivating for 4 days with a high cell density, the supernatant was separated from the residue by centrifuging the culture medium. The supernatant obtained contained 102000 U/l of recombinant lipase/acyltransferase with a specific activity of 80 U/mg of protein. Concentration by ultrafiltration with 10000 kD cut-off membranes produced an enzyme concentration of 830000 U/l with a specific activity of 150 U/mg.

Example 5: Expression of a modified (His-tagged) lipase/acyltransferase in *Saccharomyces cerevisiae*

In order to express the desired modified nucleic acid sequence of SEQ ID NO: 3, made possible by fusing 6-his-peptide to the C-terminal end of the sequence for the polypeptide of SEQ ID NO: 2, DNA was initially partially hydrolyzed with the restriction endonuclease *Bam*HI. Primers were constructed using PCR, which primers contained the nucleic acid of SEQ ID NO: 1. The following primer pairs, which made possible an extension of the nucleic acid sequence with 6 histidine codons, were used (start and stop codons are underlined; *Bam*HI restriction site is shown in bold; His codons are in italics):

forward 5'-CTC**GGATCC**ATGCGTTACTTTGCTATTGC

reverse 5'-CAC**GGATCC**TTA*ATGATGATGATGATGATG*AAAAGCAAAACGTTCCAACTTGAGCAATCC

The following time/temperature program was followed for PCR amplification: denature 5 min at 95°C; then 30 cycles of 1 min at 95°C, 1 min at 50°C, 1 min at 72°C; and the final step, 10 min at 72°C.

The fragments from PCR were digested with the restriction endonuclease *Bam*HI and then ligated into the vector pVT100-U cleaved with the restriction endonuclease *Bam*HI, to produce a plasmid. The vector pVT100CpLIP2His of **Figure 3** (integrative plasmid) was obtained. Transformation of *Saccharomyces cerevisiae* W303-1a and gene expression was carried out as described in Example 3.

The selected transformant was cultivated in shaker flasks at 28°C in YPD medium (YPD = 10 g/l yeast extract [Difco], 20 g/l Bacto peptone [Difco], 60 g/l glucose, 150 mg/l leucine, 100 mg/l adenine, 100 mg/l histidine and 100 mg/l tryptophan). The culture medium was harvested after 36 hours and the culture solution supernatant was separated from the residue by centrifuging. The supernatant contained 3100 U of recombinant his-tagged lipase/acyltransferase per liter and had a specific activity of 0.25 U/mg of protein. Ion-chelating properties were exploited for single-step purification. Ni-nitrilo triacetic acid agarose affinity chromatography gel from Qiagen was used in accordance with the manufacturer's instructions. 26% of the enzyme was obtained with a specific activity of 150 U/mg of protein.

SEQUENCE LISTING

Claims

1. Polypeptides with lipase/acyltransferase activity with an amino acid sequence which is at least 49%, preferably 80% identical to the amino acid sequence given in SEQ ID NO: 2.

2. Polypeptides with lipase/acyltransferase activity with an amino acid sequence which is 96% identical to the amino acid sequence given in SEQ ID NO: 2 in positions 190 to 390.

3. Polypeptides with lipase/acyltransferase activity with an amino acid sequence which is identical to the amino acid sequence given in SEQ ID NO: 2.

4. Polypeptides according to one of claims 1 to 3, **characterized in that** they are present glycosylated.

5. Polypeptides according to claims 1 to 3, **characterized in that** they are joined to a further peptide.

6. Polypeptides according to claim 5, **characterized in that** the further peptide is a marker, preferably his-tag.

7. Polypeptides with an amino acid sequence which has at least 49%, preferably 80% identity with the amino acid sequence given in SEQ ID NO: 4.

8. Polypeptides with an amino acid sequence which is 100% identical to the amino acid sequence given in SEQ ID NO: 4.

9. Polypeptide fragments or polypeptides obtainable by deletion mutation with lipase/acyltransferase activity according to one of claims 1 to 8.

10. Polypeptides obtainable by deletion mutation or chimeric polypeptides with lipase/acyltransferase activity, at least one part of which consists of a polypeptide which is identical to a polypeptide or fragment according to one of claims 1 to 9.

11. Derivatives of a polypeptide with lipase/acyltransferase activity according to one of claims 1 to 10.

12. Polypeptides with lipase/acyltransferase activity which have at least one antigenic determinant in common with one of the polypeptides or derivatives named in claims 1 to 11.

13. Polypeptides according to at least one of claims 1 to 12, **characterized in that** they can be obtained naturally from a microorganism.

14. Polypeptides according to claim 13, **characterized in that** the microorganisms are eukaryotic fungi, preferably yeast fungi.

15. Polypeptides according to claim 13, **characterized in that** they can be obtained from microorganisms selected from the group which is formed by *Candida parapsilosis, Candida antarctica (Trychosporon oryzae, Pseudozyma antarctica), Candida glabrata, Candida albicans, Candida maltosa, Candida tropicalis, Candida viswanathil, Issatchenkia orientalis (Candida krusei), Kluyveromyces marxianus (C. kefyr, C. pseudotropicalis), Pichia guilliermondii (Candida guilliermondii), Geotrichum candidum, Fusarium solani* and *Aeromonas aerophila*.

16. Nucleic acids coding for a polypeptide with lipase/acyltransferase activity, the nucleotide sequences of which are 100% identical to the nucleotide sequence given in SEQ ID NO: 1, in particular over the region which corresponds to the amino acids 190 to 390 according to SEQ ID NO: 2.

17. Nucleic acids coding for an amino acid sequence which has at least 49%, preferably 80% identity with the amino acid sequence given in SEQ ID NO: 2.

18. Nucleic acids coding for an amino acid sequence which is at least 96% identical to the amino acid sequence given in SEQ ID NO: 2 in positions 190 to 390.

19. Nucleic acids which code for one of the polypeptides or derivatives named in claims 1 to 15.

20. Nucleic acids coding for a polypeptide with lipase/acyltransferase activity, the nucleotide sequence of which is identical to the nucleotide sequence given in SEQ ID NO: 3.

21. Nucleic acids coding for an amino acid sequence which has at least 49%, preferably 80% identity with the amino acid sequence given in SEQ ID NO: 4.

22. Organism which forms one of the proteins or derivatives named in claims 1 to 15 or contains nucleic acids coding for same.

23. Organism according to claim 22, **characterized in that** it is a microorganism, preferably a yeast fungus.

24. Organism according to one of claims 22 and/or 23, selected from the group which is formed by *Candida parapsilosis, Candida antarctica (Trychosporon oryzae, Pseudozyma antarctica), Candida glabrata, Candida albicans, Candida maltosa, Candida tropicalis, Candida viswanathil, Issatchenkia orientalis (Candida krusei), Kluyveromyces marxianus (C. kefyr, C. pseudotropicalis), Pichia guilliermondii (Candida guilliermondii), Geotrichum candidum, Fusarium solani* and *Aeromonas aerophila.*

25. Vector which contains a nucleic acid named in claims 16 to 21, which codes for one of the polypeptides or derivatives named in claims 1 to 15.

26. Cloning vector according to claim 25.

27. Expression vector according to claim 25.

28. Cell which contains a vector according to one of claims 25 to 27.

29. Transformed host cell which expresses or can be incited to express one of the polypeptides or derivatives named in claims 1 to 15, preferably using an expression vector according to claim 27.

30. Transformed host cell according to claim 29, containing a nucleic acid which codes for an amino acid sequence which has 100% identity with the amino acid sequence given in SEQ ID NO: 2.

31. Transformed host cell according to claim 29 and/or 30, containing a nucleic acid which codes for an amino acid sequence which has at least 49%, preferably 80% identity with the amino acid sequence given in SEQ ID NO: 2.

32. Transformed host cell according to claim 29, containing a nucleic acid which codes for an amino acid sequence which has 100% identity with the amino acid sequence given in SEQ ID NO: 4.

33. Transformed host cell according to claim 29 and/or 30, containing a nucleic acid which codes for an amino acid sequence which has at least 49%, preferably 80% identity with the amino acid sequence given in SEQ ID NO: 4.

34. Transformed host cells according to one of claims 29 to 33, **characterized in that** the host cells to be transformed are host cells from microorganisms.

35. Transformed host cells according to one of claims 29 to 34, **characterized in that** the host cells to be transformed are host cells from microorganisms which are selected from the group formed by *Candida parapsilosis, Candida antarctica (Trychosporon oryzae, Pseudozyma antarctica), Candida glabrata, Candida albicans, Candida maltosa, Candida tropicalis, Candida viswanathil, Issatchenkia orientalis (Candida krusei), Kluyveromyces marxianus (C. kefyr, C. pseudotropicalis), Pichia guilliermondii (Candida guilliermondii), Geotrichum candidum, Fusarium solani* and *Aeromonas aerophila.*

36. Process for the preparation of a polypeptide according to at least one of claims 1 to 15, using a nucleic acid which codes for an amino acid sequence which has at least 49%, preferably 80% identity with the amino acid sequence given in SEQ ID NO: 2, and/or using a vector according to one of claims 25 to 27 and/or using a transformed host cell according to one of claims 29 to 35 or using a cell which forms this naturally, in particular a cell from an organism according to claims 22 to 24.

37. Use of natural and/or recombinant microorganisms containing a nucleic acid for the preparation of a polypeptide according to at least one of claims 1 to 15.

38. Use of a nucleic acid according to claims 16 to 21 and/or use of amino acid sequences which have at least 49%, preferably 80% identity with the amino acid sequence given in SEQ ID NO: 2 for the discovery of new acyltransferases.

39. Use of a nucleic acid according to claims 16 to 21 and/or use of amino acid sequences which have at least 49%, preferably 80% identity with the amino acid sequence given in SEQ ID NO: 4 for the discovery of new acyltransferases.

40. Use of polypeptides according to claims 1 to 15 as catalysts in acyl transfer reactions, in particular in reactions which are selected from the group which is formed by alcoholysis of esters, alcoholysis of thioesters, thiolysis of esters, aminolysis of an ester with hydroxylamines or hydrazines; reaction of an ester with hydrogen peroxides and enantioselective synthesis of esters, thioesters, lactones by alcoholysis.